3. The applicant submits that the original specification has been amended to correct the informalities noticed.

Response to Objection of the Claims 1-36

4. The applicant submits that the original claims 1 and 22 are amended respectfully to correct the informalities noticed by the Examiner.

Response to Rejection of Claims 1-36 under 35USC112

5. The applicant submits that the amended claims 1 to 36 containing subject matter which was described in the amended specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, as pursuant to 35USC112, first paragraph.

6. The applicants respectfully submit that, although the Examiner alleges that the claims 1 to 36 containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention, the Examiner does not clearly indicate which portion(s) of the disclosure in the specification fail to illustrate how to make and use the claimed invention so as to support the claims 1 to 36 of the instant invention. The only mention in the Office Action is in page 4 that the Examiner alleges the specification provides only limited guidance. The applicants respectfully submit that the disclosure of a patent specification is for someone who skilled in that art to learn from the claimed invention.

7. Although the applicants believe that the disclosure of the specification contains subject matter to enable one skilled in the art to make and/or use the claimed invention, the applicants amend the specification to include a more detailed disclosure to further clarify the rationales and best experimental conditions of the instant invention.

8. The Example 1 describes the prevention of RNA degradation in cells. The Examples 2-4 are directed to each step of the second preferred embodiment (Figures 2 and 3) for a very detailed description, while the Example 5 is drawn to the first preferred embodiment (Figure 1) published in *Nucleic Acid Res.* 27: 4585-4589 (1999). Although both practical procedures have been tautologically shown in Examples and Figures, the

scientific theory of each step does not complicate the applicability of our current invention. In fact, one of its best modes is completed in a microtube with only one change of buffering conditions as shown in Example 5 (Figure 1). Therefore, although certain preferred embodiments of the present invention have been described in detail, the purpose of such disclosure is to demonstrate its practical modes under best conditions but not to challenge its real performance.

9. The Examiner's citation of Carrico's hybridization conditions (US Patent 5,200,313) is even a proof of our success in fulfilling the requirements of 35 U.S.C. 112. For example, (1) the first reverse transcription with poly(dT)-containing primer for the purity of cDNA preparation from poly(A)-mRNAs (Examples 2 & 5), (2) the use of T7 promoter-linked oligo(dG or dC)-primers for the higher G-C content annealing at higher temperatures (Examples 4 & 5), (3) the use of promoter-linked oligo(dG or dC)$_{10\sim12}$mers (annealing $T^0 > 30^0C$) for specific cDNA double-stranding (Examples 3 & 5), (4) the special design of our RT&T buffer for proper ionic strength in reactions, (5) optimal annealing temperatures at 30~55^{0}C depending on the G-C content of the promoter-linked primers (Examples 3, 4 & 5), (6) optimal primer concentration (0.5~1.0 micromolar in Examples 2-5), (7) heat denaturation (Examples 4 & 5) and (8) proper annealing time (1~10 min in Examples 2-5) depending on the primers. All pertinent information was described in Examples in detail, matching the required conditions for hybridization. This evidence proves that the instant invention has provided enough experimental detail for the success of the present invention.

10. The feasibility of the instant invention is at the same practical level as Eberwine's invention. The novelty of Eberwine's patent is based on the use of a promoter-linked poly(dT) primer for antisense RNA (aRNA) fragment generation, while ours is the use of a promoter-linked poly(dG/dC) primer mixture for messenger RNA (mRNA) cycling amplification, more preferably for "full-length" mRNA library amplification. Both inventions involve some conventional enzyme reactions such as reverse transcription, cDNA double-stranding and in-vitro transcription. Although the instant invention conveys more specific primer-annealing due to improvements of the terminal transferase tailing reaction, the basic works of the aforementioned reverse transcription, cDNA double-stranding, in-vitro transcription and tailing reaction are all standard procedures as described in Sambrook's *Molecular Cloning* book (Cold Spring Harbor Laboratory Press, 1989) with little modification. However, the special improvements and

modifications of the instant invention will be further highlighted in the amended specification for better demonstration, including rationales and optimal buffer conditions for different enzymatic reactions.

11. In brief, the claims 1 to 36 of the instant invention are drawn to a method of generating amplified messenger RNAs (mRNAs) from mRNA templates, contacting it with first poly(dT)-containing primers to generate first-strand cDNA from the mRNA, adding a polynucleotide sequence to the tail of the cDNA, generating a double-stranded cDNA from the polynucleotide-tailed cDNA using second promoter-containing primers annealed to the cDNA tail, and generating mRNAs from the promoter-containing double-stranded cDNA (FIG.1). The whole procedure of this invention is as simple as a biochemical kit and its cycling amplification steps(d)-(f) can be performed in a microtube without changing anything as shown in Example 5 as well as in our publication (Lin et.al, *Nucleic Acid Res.* 27: 4585-4589, 1999).

12. It has been well known that the generation of full-length mRNAs is a bottleneck of current cDNA library research at the single-cell scale which is especially important for cancer research in vivo (Embleton et.al, *Nucleic Acid Res.* 20: 3831-3837, 1992.; Lin, 1999). Previous methods failed to preserve the complete 5'-end of an RNA sequence for amplification due to the lack or loss of specific primer binding sites (Eberwine's invention) or due to the failure of the PCR reaction through polyG-C tails (Sambrook's protocols). The instant invention overcomes this bottleneck by designing special promoter-containing primers to form the most 5'-end of a cDNA template in the sense orientation for full-length mRNA generation and using the in-vitro transcription reactions (not PCR-based reactions) to amplify these full-length mRNAs without the drawbacks of PCR. Therefore, the instant invention advantageously provides more flexibility to the generation of either pure full-length mRNAs (Figure 1) or double-stranded RNA (mRNA/aRNA) mixtures (Figure 2), ready for a variety of biochemical applications such as full-length mRNA/cDNA preparation, probe preparation, in-vitro translation and gene knockout analysis (RNA interference).

The Cited but Non-Applied References

13. The cited but not relied upon references have been studied and are greatly appreciated, but are deemed to be less relevant than the relied upon references.

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of the objection and rejection are requested. Allowance of claims 1 to 36 at an early date is solicited.

Respectfully submitted,

Raymond Y. C. Chan
Reg. Nr.: 37,484
1050 Oakdale Ave.,
Arcadia, CA 91006-2222
Tel.: 1-626-571-9812
Fax.: 1-626-571-9813

CERTIFICATE OF MAILING

Express Mail Label # EL 740910658 US **; Date of Deposit** 01/02/2001

I hereby certify that this corresponding will be deposited with the United States Postal Service by Express Mail, postage prepaid, in an envelope addressed to "Box Amendments, Commissioner of Patents and Trademarks, Washington, DC 20231" on the date below.

Date: January 02, 2001

Signature:
Person Signing: Raymond Y. Chan